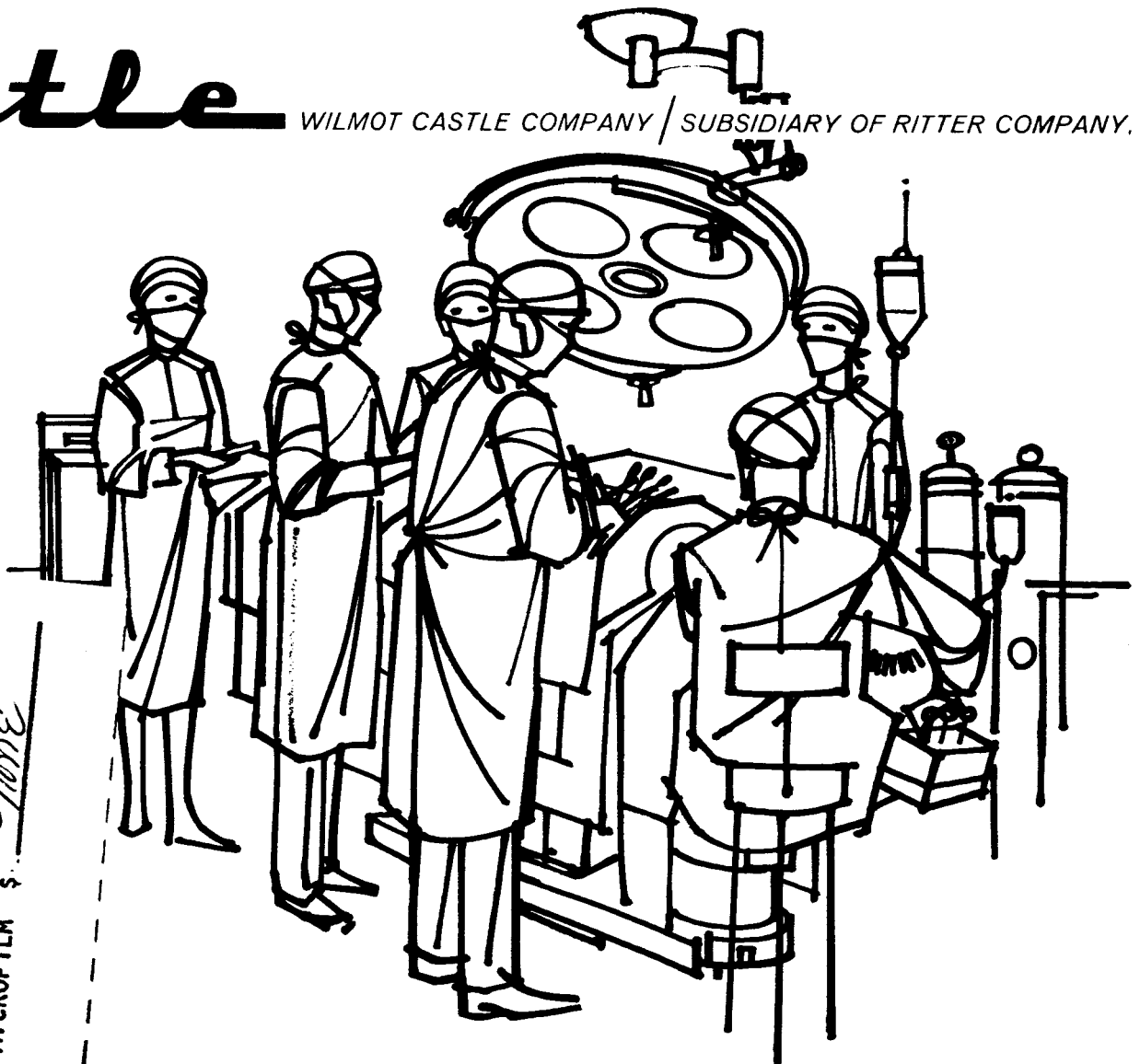


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STUDIES FOR STERILIZATION

OF

SPACE PROBE COMPONENTS

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PROGRESS REPORT NO. 2

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

CONTRACT NASw-879

December 1st, 1963 - March 1st, 1964

RESEARCH LABORATORIES
WILMOT CASTLE COMPANY
ROCHESTER, NEW YORK

STATUS OF REPORTS:

This Progress Report covers the research performed from December 1st, 1963, to March 1st, 1964, under NASA Contract NASw-879 by the Research Laboratories of the Wilmot Castle Company.

The first Progress Report on this Contract was issued December 1st, 1963.

STATUS RESEARCH ACTIVITIES:

The research activities for this period have been concerned with the specific work objectives as previously outlined. These studies have been continued without any unusual problems.

CURRENT RESEARCH ACTIVITIES REPORTED ON HEREIN INCLUDE:

The current research activities which were continued or initiated during the period covered by this report include:

I. Studies on the dry heat resistance of microorganisms:

1) Entrapped from air samples:

- a) on membrane filters,
- b) in or on glass petri plates, microscope slides, and jars and,
- c) in liquid impingers, then concentrated on membrane filters and dried.

2) Added to sterile kaolin,

3) In various gaseous atmospheres,

4) In activated carbon

II. Studies which were initiated on the sterilization of components:

1) Objects which have accumulated natural contamination in an industrial manufacturing area,

2) Techniques for determining levels of contamination as well as possible sterility of components and,

3) Commercial components in the temperature range 100-135°C.

RESULTS OF CURRENT RESEARCH ACTIVITIES:

I. Studies on the dry heat resistance of microorganisms:

1) Entrapped from air samples:

a) On membrane filters

Microorganisms have been collected on membrane filters, (Millipore type HA)¹ the filters then cut in half; one half was assayed for level of organisms and the other half treated in dry heat (in test tubes in the cylindrical aluminum block units or in a hot air oven with forced circulation) for various times at temperatures in the range 115-135°C. The heat treated filter-halves were then assayed for sterility by adding sterile tryptone glucose yeast extract broth and incubated for no less than two weeks at 32°C. After that time all tubes not showing evidence of growth were subcultured by streaking onto plate count agar slants.

Results of the work which has been completed on this phase are summarized in Table 1. Very low levels of viable organisms were collected on these filters and brief treatment cycles are adequate to sterilize them.

It should be mentioned that impactor sieve sampler²

¹ Millipore Filter Corporation, Bedford, Mass.

² Technical Development Laboratories, Communicable Disease Center, U. S. Public Health Service, P. O. Box 769, Savannah, Georgia

and impinger³ samples indicate that much higher levels of viable organisms are present in the same environmental atmosphere. Additional studies are currently under way to evaluate this aspect.

3 Midget impinger, Gelman Instrument Co., Ann Arbor, Michigan.

TABLE 1

Preliminary Results of the Recovery of Microorganisms Collected on Membrane Filters¹ From a Microbiological Laboratory and The Heat Resistance of Such Samples.

Sampling period (days)	Volume of Air Sampled (cu. ft.)	Number of Samples	Number of Viable Organisms Per Filter-Half ²	Average	Range	Results of Heat Treatment of Organisms on Filter-Halves ³
1	605	43	85		10-1200	120°C Sterile after 1 hr 135°C Not sterile after 15 min
3	1815	17	340		10-2072	115°C Not sterile after 1 hr; but sterile after 2½ hr
4	2420	9	77		40-200	115°C Not sterile after 1 hr
13	7865	12	218		72-408	115°C Not sterile after 1 hr; but sterile after 2 hr

1 Filters employed in this series of tests were Type HA, Millipore Filter Corporation, Bedford, Mass.

2 Aerobic mesophilic spore population as assayed after a heat-shock of 65°C for 30 min and plated on plate count agar

3 Results are for sterility tests employing tryptone glucose yeast extract broth at an incubation temperature of 32°C for a period of not less than two weeks.

I 1)

b) in or on glass petri plates, microscope slides, and glass jars:

Studies are under way to determine the level of microbial contamination that accumulates naturally on surfaces from fallout and to evaluate what dry heat cycles are required to sterilize such items on which microorganisms have accumulated.

Three tests employing sterilized glass petri plates, microscope slides and 5 oz. glass baby food jars are currently under way in which these items are being exposed and allowed to collect whatever microorganisms they will in various areas of the laboratory for periods up to six months. The level of contamination on these items will be evaluated and replicates will be treated by various dry heat cycles in an attempt to evaluate the natural resistance of the microbial populations obtained.

I 1)

c) in liquid impingers, then concentrated onto membrane filters and dried:

In an attempt to collect the maximum levels of contaminants from the atmosphere, impinger samples are being drawn and the collected organisms are concentrated onto membrane filters. The filters are placed in desiccator jars to dry. Samples will then be assayed and replicates exposed to various heat treatments in a further attempt to elaborate what cycles may be required to sterilize such populations.

I 2) added to sterile kaolin:

In an attempt to determine what effect the chemical-physical environment of the organism on a carrier might have on the resistance of organisms to dry heat treatment, experiments have been initiated employing spores of Bacillus subtilis var. niger added to sterile kaolin.¹ The kaolin was sterilized by dry heat at 160°C for 12 hrs.

It is intended to inoculate untreated and treated bacterial spores into untreated and treated kaolin. The heat resistance of these preparations when exposed to dry heat treatments will be investigated.

¹ Technical grade #2240, J. T. Baker Chemical Co., Phillipsburg, N. J.

I. 3) in various gaseous atmospheres:

Experiments have been initiated to determine the comparative resistance of bacterial spores to dry heat in or under various gaseous atmospheres including dry air, nitrogen or helium. A system has been devised which allows the heated gas to pass through a series of U-tubes with supports which, for the initial work, hold paper strips. The gas is heated and bled through the system at a rate of 2.54 CFH. The entire system is held in constant temperature oil bath.

The apparatus allows observation on the effect of a hot flowing gas on bacterial spores. The spores can hence be treated with a continuous flow or in a stagnant or non-circulating atmosphere.

Initial studies are under way so as to compare the effectiveness of air and nitrogen under each of the above conditions. Additional work is in progress and should give more positive conclusions by the time of the next report.

I. 4) In activated carbon:

Results of experiments on the resistance of bacteria and bacterial spores in activated carbon have been reported in Progress Report No. 1 on this contract. The results reported here are on another brand of activated carbon--Darco G-60¹, samples of which have been reported to contain microorganisms which survived extremely severe thermal treatments.

Levels of organisms in Darco G-60 have been approximated from a freshly obtained sealed can by employing a tube-dilution (growth or no-growth) technique as well as the standard plate count technique. The results of these assays are given in Table 2.

Samples of Darco G-60 activated carbon (50 mg/tube) have been treated:

- a) with dry heat at temperatures of 120°C and 135°C for various times.
- b) with saturated steam in an autoclave at 15 psi at 121°C for 15, 30, and 45 min.

The preliminary results of the effect of these treatments are (see Tables 3 and 4)

- a) that growth was observed in only 1 sample out of 34 for 3 hr or longer at 120°C in dry heat, when cultured in thioglycollate broth at 32°C and 55°C. No growth was observed in

¹ Atlas Chemical Industries, Inc., Wilmington 99, Delaware.

samples treated for 3 hr and $\frac{1}{2}$ hr at 135°C in dry heat when cultured in thioglycollate broth at 32°C and 55°C, respectively. (see Table 3)

- b) that growth was observed in only 2 of 40 samples treated for 45 min in saturated steam at 121°C (15 psi) when cultured in thioglycollate broth at both 32°C and at 55°C respectively. Growth was observed in a significantly greater percentage of samples exposed for 30 and 15 min. (see Table 4)

- I. 4) All of the treated samples were cultured for at least ten days, after which time all tubes were subcultured by streaking and stab inoculation on plate count agar slants. These subcultures were incubated at the same temperatures as the respective original cultures. Only one subculture streak out of 160 tubes gave a result differing from the original culture.

TABLE 2

Levels of Bacterial Population in Darco G-60 Activated Carbon

<u>Population Assayed</u>	<u>Range of Population¹</u>		
Mesophilic aerobes ²	2×10^2	-	8×10^3
Mesophilic aerobes (spores) ³	7×10^3	-	8×10^3
Mesophilic anaerobes	1.5×10^1	-	1×10^3
Mesophilic anaerobes (spores)	3×10^1	-	3.5×10^2
Thermophilic aerobes	3×10^3	-	1×10^4
Thermophilic aerobes (spores)	3×10^3	-	3.5×10^3
Thermophilic anaerobes	2×10^3	-	3.5×10^3
Thermophilic anaerobes (spores)	3.5×10^3	-	5×10^3

-
- 1 Results are for assay of five 0.1 g samples plated in triplicate at each of three dilutions. Assays for aerobic populations were plated on plate count agar. Assays for anaerobic populations were run in thioglycollate agar in oval tubes.
 - 2 Counts from nonheat shocked samples.
 - 3 Counts from samples heat shocked (20 ml of original dilution) at 65°C for 30 min.

TABLE 3

Observations on the Times Required to Sterilize 50 mg Samples of Darco G-60
Activated Carbon with Dry Heat Treatments

<u>Treatment Temperature</u> <u>Temperature of Incubation</u>	<u>at 120°C</u>		<u>at 135°C</u>	
	32°C	55°C	32°C	55°C
<u>Treatment Time</u>				
10 min.			3/3	2/3
15 min.			2/3	0/3
30 min.	3/3*	3/3		
1 hr.	2/3	1/3	9/12	0/12
3 hr.	1/12	0/12	0/6	
6 hr.	0/4			
12 hr.	0/6			

* Results are given as number of samples showing growth over the total number of samples treated. Sterility tests were performed by adding sterile thioglycollate broth to the tubes containing the samples after treatment. All samples were incubated for at least 10 days and then sub-cultured into sterile slants of plate count agar.

TABLE 4

Observations on the Times Required to Sterilize 50 mg Samples of Activated Carbon
with Moist Heat at 121°C (15 lbs pressure)

Treatment Time	15 min	30 min	45 min
Temperature of Incubation	<u>32°C</u> <u>55°C</u>	<u>32°C</u> <u>55°C</u>	<u>32°C</u> <u>55°C</u>
Position of tubes	Horizontal	Horizontal	Horizontal
	9/20*	0/20	1/20
	17/20	2/20	1/20
	20/20	7/20	1/20

* Results are given as number of samples showing growth over the total number of samples treated and are for sterility tests performed by adding sterile thioglycollate broth to the tubes containing the samples after treatment. All samples were incubated for at least two weeks and then subcultured into sterile butts of plate count agar.

II. Studies which were initiated on the sterilization of components

- 1) objects which have accumulated natural contamination in an industrial manufacturing area:

The objects picked up from an industrial manufacturing area included greasy or oily screws, nuts and bolts, metal scraps, washers, nails, pieces of tubing or conduit, etc. It was assumed that such items would have collected a mixed flora of microorganisms from handling by personnel and from aerial fallout, etc. Representative items were assayed to determine what the frequency and range of the natural contaminants were. The results are given for 17 items in Table 5. Most items had a very low bacterial population.

Another set of items was treated in a hot air oven for various times at 135°C and then assayed for sterility in tryptone glucose extract broth employing conventional aseptic transfer procedures. Few items showed contamination after even the shortest treatment (Table 6). After the incubation period was completed, 100 spores of Bacillus subtilis var. niger were added to all culture vessels showing no growth to substantiate whether or not organisms were inhibited by the presence of the item in the culture broth and that the medium was capable of

supporting growth of organisms if any were present.

Several of these tubes did prevent the inoculum from growing.

TABLE 5

Observations On The Levels Of Microbial Contamination On Items Selected From An Industrial Manufacturing Area

Level of Microorganisms Per Item	Number of Items Harboring			<u>Molds</u>
	<u>Bacteria</u> Heat Shock ¹	<u>Bacteria</u> No Heat Shock		
None	1	1		4
1-20	10	8		5
21-40	4	3		--
41-60	0	3		--
>100	2	2		1

¹ 5 ml aliquots of rinse solution were heat shocked at 65°C for 30 min then plated.

TABLE 6

Observations On The Times Required To Sterilize Items Selected From An Industrial Manufacturing Area In Dry Heat at 135°C.

Exposure Time	<u>1.75 hr</u>	<u>3 hr</u>	<u>6 hr</u>
Number of Items Not Sterilized	4	4	2*
Total Number of Items Treated	41	65	26

* These two items (plus 2 others) were cultured in baby food jars. All the other items (128) were cultured in screw cap tubes. None of 18 control jars containing sterile media showed contamination after two weeks incubation even when the lids were left ajar for as long as 48 hrs prior to incubation.

- II 2) techniques for determining levels of contamination as well as possible sterility of components:

It is well understood that in order to reliably establish levels of contamination as well as absence of contamination (i.e. sterility) all work must be performed in an uncontaminated atmosphere. The usual means of obtaining such an atmosphere is to place a barrier around the objects to be evaluated and to sterilize the interior of that barrier and maintain sterility in the enclosed atmosphere. The conventional means of accomplishing this is to employ airtight plastic chambers such as are used in the germ-free animal program. The techniques for the use of such isolator systems have been reported on by other groups as regards the testing or evaluation of microbial contamination inside electronic components. The conventional isolator has been employed to test several components at one time and the fact remains that if contamination is found in one of the first items tested, and in others later on, then nothing can be concluded as regards the latter items. In order for the results to be meaningful, each item (or component) must be evaluated individually and with the necessary individual controls.

It is for this reason, that we are currently evaluating the use of smaller flexible isolator systems adapted and

modified for this task. The isolators are described in the attached diagrams. (See Figures 1-3)

The status of this phase is proceeding due to anticipation of receipt of specific components from NASA headquarters. Such a technique will allow more components to be evaluated individually and reliably both as to levels of microbial contamination (external as well as internal) and/or the sterility of the item.

The results on this phase of the work will be included in the next progress report.

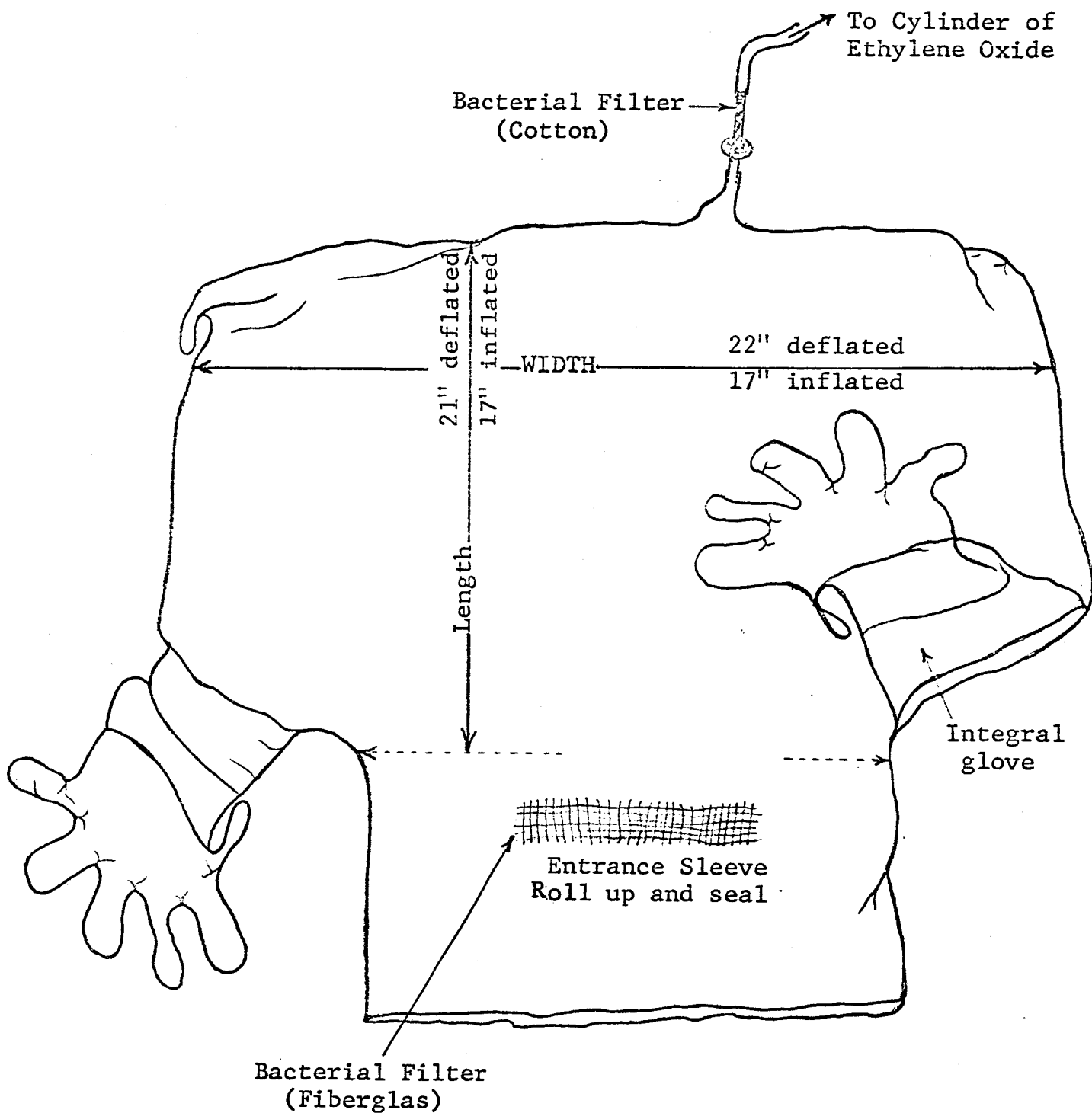


FIGURE 1. I²R GLOVE BAG MODIFIED FOR STERILE ASSAYS OF COMPONENTS.

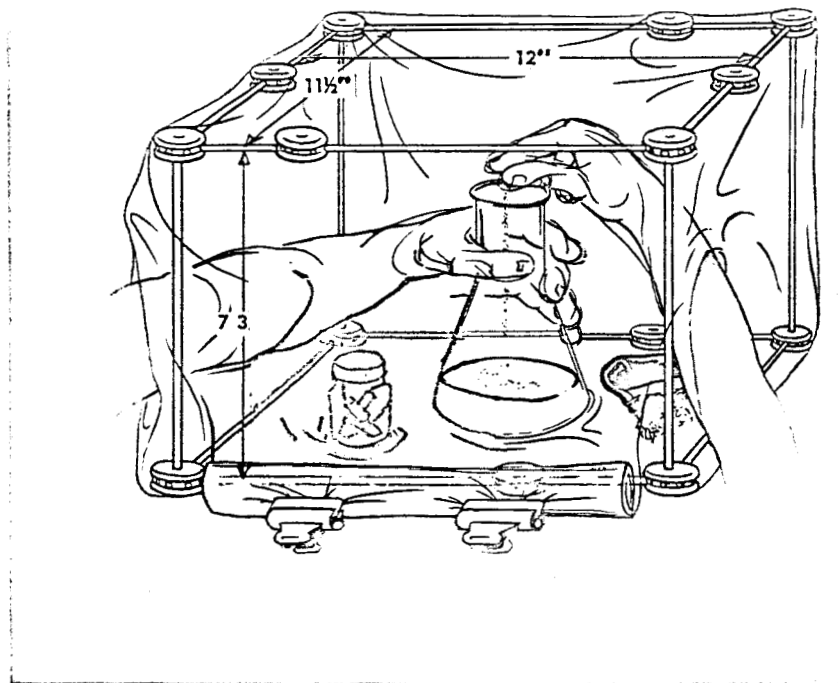


FIGURE 2. I^2R GLOVE BAG WITH RIGID FRAMEWORK.

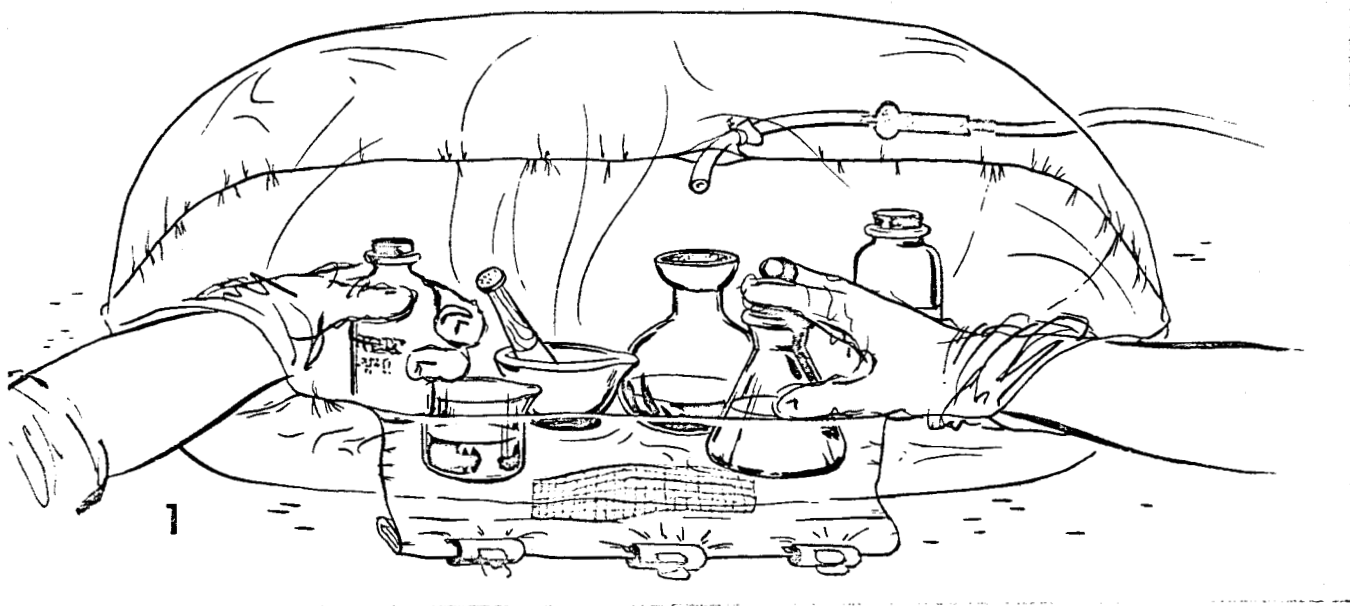


FIGURE 3. I^2R GLOVE BAG INFLATED WITH AIR OR INERT GAS.

II 3) Commercial components in the temperature range 100-135°C:

Several electronic components including resistors, a capacitor and some printed circuit boards (both rigid and flexible) were assayed aseptically for the level of external microbial contamination and then placed in glove bags² to be broken up for evaluation of the level of internal contamination. Some of the components were untreated, while others were treated with dry heat at temperatures of 115°C and 125°C for various times.

The components which were to be assayed internally, as well as all the other necessary equipment and the glove bags and their atmosphere were sterilized by admitting gaseous ethylene oxide. A concentration of no less than 500 mg of ethylene oxide per liter of space was employed in an overnight exposure (12 hr minimum). The following day the glove bags were aerated with air passed through cotton packed drying tubes for several hours prior to initiation of the assay.

The assays were performed crushing the components with pliers and placing the pieces into sterile water blanks, then removing these bottles from the isolator, sonicating them and plating all of the rinse water with plate count agar.

The results indicate that a very low number of bacteria

² Model R-17-17, Instruments for Research and Industry, Cheltenham, Pa.

(0-25 per component) were present on the exterior as well as on the interior of most of the components assayed in this manner. Since counts of numbers of organisms in this range are not very reliable and since the plating was performed on the open bench, even though strict aseptic procedures were employed, the results are reported in Table 7 as the number of components showing greater than two colonies per plate (a maximum control plating level) as a fraction of the total number tested.

The results of the entire series of heat treatments are not complete at this date but do indicate that the brief dry heat cycles employed were not adequate to sterilize the exterior let alone the interior of most of the components. If the results of the levels of contamination of these components is any indication of what might be found normally on such items, then extreme care must be employed in any such evaluation of the levels of contamination.

It is anticipated in the next quarter that selected components are to be supplied through NASA for which evaluation of levels of contaminating microorganisms are to be performed. The techniques practiced on the components evaluated herein will be expanded and improved upon.

TABLE 7

Preliminary Results of Bacterial Assays Performed On Commercial Electronic Components After Various Dry Heat Treatments

Type of Component	Dry Heat Treatment		Result of Bacterial Assay ¹	
	Temperature (°C)	Time (hr)	Exterior	Interior
Resistor	None		6/6	4/6
	115	6	3/6	4/6
	115	12	6/6	5/6
	125	6	3/6	3/6
Capacitor (oil filled)	115	12	1/1	1/1
Printed circuit (rigid) board ²	115	2	5/10	(Not completed)
	115	4	1/12	
	115	12	4/10	
	125	6	3/12	
(flexible)	115	2	5/6	(Not completed)
	115	4	1/6	
	115	12	1/2	
	125	6	1/6	
Resistor (SAGE type, wire wound)	115	12	1/4	2/4

1 Results are given as number of components producing more than two colonies per component as a fraction of the total number assayed.

2 A whole circuit board (6 x 3 in) was cut into smaller sections (2 x 1/4 in) and these were considered separate items.

RESEARCH ACTIVITIES FOR THIRD QUARTER

The research activities which will be concluded, continued, or initiated during the third quarter of the current contract include:

1 - Studies on the dry heat resistance of microorganisms:

- a) Carried on sterile kaolin, which have been treated in an attempt to alter the chemical-physical environment of the organisms and/or material.
- b) In a heated gaseous atmosphere, both non-circulating and circulating air and nitrogen, and if possible helium.
- c) Encapsulated in solid materials such as plaster of paris and dental die materials.

2 - Studies on the sterilization of components including:

- a) Commercial components (supplied by NASA) in the temperature range 100-135°C, as to levels of natural contamination and cycles required to sterilize such levels of contamination.

Reported Submitted: March 1st, 1964

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NASA Contract NASw-879